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(54) Title: A KIT FOR QUALITATIVE AND QUANTITATIVE ANALYSIS OF ANTIGENS, ANTIBODIES AND/OR MICROORGANISMS OR OTHER CELLS

(57) Abstract

The reagents in the kit are included in an absorbant material, with the reagents in dry alternatively lyophilized form. The reagents are arranged in one of two ways. In Arrangement I the order of the reagents is (1) purified complexes of enzyme and antibodies against the antigen to be detected; (2) antigen immobilized in the absorbant material; and (3) substrate to the enzyme. In Arrangement II the order of reagents is (1) purified complexes of enzyme and antigen, which antigen is antigenically related to the antigen to be detected and which antigen is immobilized in the absorbant material; and (3) substrate to the enzyme. Arrangement I can also be used to detect antibody. In both cases, the test fluid is allowed to pass, by capillary forces, from step (1) to step (3). The presence of the antigen (antibody) to be tested for is thereby detected by the occurrence of a colour reaction at (3), due to the splitting of the substrate. The kit therefore allows a very simple procedure for detection of a compound without washing steps etc. usually included in the ELISA-technique. The invention also creates possibilities for quantitation by instrumental reading of the colour change at step (3).

A KIT FOR QUALITATIVE AND QUANTITATIVE ANALYSIS OF ANTIGENS, ANTIBODIES AND/OR MICROORGANISMS OR OTHER CELLS

The present invention concerns a kit for qualitative and quantitative analysis of antigens, antibodies and/or microorganisms or other cells.

The term "antigen" as used herein means not only substances which are capable by themselves of provoking the production of antibodies in animals, i.e. immunogens, but also substances, sometimes called haptens, which, after conjugation with a carrier molecule, become capable of provoking the production of specific antibodies.

Our international application under the Patent Cooperation Treaty of number PCT/SE86/00309, describes a method for quantitative or qualitative analysis of a compund, consisting of antigens, antibodies, microorganisms or other cells. As far as concerns antigens, antibodies or small microorganisms these compounds are bound to solid material (particles) whereas bacteria, fungi or other cells can be analyzed directly. The antigens are then brought into contact with enzyme-labelled antibodies against the antigens, or complexes of unlabelled antibodies against the antigens, and enzyme-labelled anti-bodies. The mixture is forced to pass a filter which retains the complex--bound smaller antigens or the cells, but allows un--bound specific enzyme-labelled antibodies (alternatively complexes of specific antibodies and enzyme--labelled anti-bodies) to pass the filter. After passage of the filter, the enzyme-labelled antibodies are brought into contact with a substrate, which is degraded by the enzyme. In such cases where a specimen is investigated for soluble small antigens, the application does also describe a method consisting of particles to

which antibodies (unlabelled) are attached and enzyme--labelled soluble specific antibodies are present together in the fluid brought into contact with the specimen to be analyzed; in this variant both antibodies are directed against the antigen to be detected but the enzyme-labelled antibody might also be replaced of an unlabelled andibody combined with an enzyme labelled anti-antibody as described above. Finally, the mixture of specimen and reagents are forced to pass the filter.

Our previous application does also comprize an apparatus for performance of the above described method. This apparatus is a capped tube which is divided in an upper chamber, a filter-chamber, and a lower chamber, each chamber being separated from the others by detachable seal-devices. The reagents are present in the upper chamber and the specimen to be investigated is applicated here. The substrate which can be degraded to colour-products is present in the lower chamber. After appropriate reaction time between specimen and reagents in the upper chamber, the fluid here is forced through the filter by low pressure applicated in the lower chamber or a stamp mechanically pressuring the fluid out of the upper chamber, through the filter, down in the lower chamber.

The apparatus can also be combined with a spectrophotometer, for quantitative determination of the colour changes.

Characteristic for the method described above is the use of Filtration of Antibodies, Separated, Specific and Enzyme-Labelled, which we abbreviate FASSEL. In contrast to known methods, these reagents are purified compounds i.e. they contain only the compound which will react with the antigen to be demonstrated. It is therefore possible to read the colour--change in the lower chamber with the naked eye. Furthermore, the colour change registered automatically is much more sensitive to spectrophotometry.

We have now invented a test kit based on this technique. The kit implies several improvements in the practical handling and method.

The method described in the previous patent application has some draw-backs:

1) The performance of the metohod comprizes a two-step procedure i.e. (a) mixture of reagents and specimen, followed by a certain incubation time, and (b) application of positive pressure in the upper chamber, or negative pressure in the lower chamber.

2) The result of the test is read as the absence of a colour change.

3) The apparatus is a bit complicated to manufacture.

Keeping in mind these problems, we have developed a new method which, while exploiting the advantages of the prior method, allows more simple performance and manufacturing and reading of the results as the occurrence of a colour change when the antigen to be detected is present in the specimen, i.e. a positive test is read as the appearance of a colour.

The present invention provides a kit which consists of an absorbant porous material including three layers of reagents arranged in one or the other of the two following orders:

ARRANGEMENT I

1) Enzyme-labelled antibodies directed against the antigen to be detected. These antibodies are distributed in the matrix in lyophilized form.

2) Antigen, fixed to particles of such a size or in such a state that they do not change their position in the absorbant material substantially when the material comes in contact with a liquid. The antigen fixed to the particles is identical with, or antigenically related to the antigen to be detected by the kit, and lyophilized.

3) Substrate for the enzyme, lyophilized.

ARRANGEMENT II

1) Enzyme-labelled antigen which is identical with, or antigenically related to the antigen to be detected by the kit.

2) Antibody fixed to particles of such a size or in such a state that they do not change their position in the absorbant material when the material comes in contact with a liquid. This reagent is lyophilized before including in the absorbant matrix (like all other antigen(antibody reagent in the present invention).

3) Substrate for the enzyme, lyophilized.

It is emphasized that the expression "lyophilized" is exchangeable in the present invention with "dry". Both arrangements function in the following manner: (a) the antigen to be detected should be present in a liquid. The liquid is introduced into the kit at the reagents under 1). The liquid is sucked through the absorbant material, from 1) towards 3). When passing the reagents under 2) the following reactions will take place: (b) In ARRANGEMENT I, antigen in the specimen to be investigated will react with the enzyme--labelled antibodies which then are prevented from reaction with the antigen-particles in 2). The antibodies will therefore continue to 3) and split the enzyme whereby coloured degradation products arise. In ARRANGEMENT II, the enzyme-labelled antigen, and unlabelled antigen present in the test-specimen will compete for the antibody in layer 2). If antigen is present in the test specimen, the enzyme-labelled antigen will therefore procede to 3) and split the enzyme whereby coloured degradation products arise.

It appears that the new invention implies considerable improvements if the reagents under 1) contain only enzyme-labelled specific antibody or enzyme-linked antigen, in the two respective arrangements, i.e. no unlabelled antigen or antibody, or enzyme not attached to these compound should not be present, although

compounds which do not interfere with antigen-antibody reactions are allowed. Thus, the improvements are:

A) The kit allows the test to be performed as a one-step method.

B) The precence of the antigen in the test-specimen is read as a positive colour change.

C) Manufacture of the kit is simple since detachable seal-devices are not required for separation of the reagents.

It should furthermore be emphasized that ARRANGEMENT I can be used for detection of the precense of antibodies in a test specimen i.e. the enzyme-labelled antibodies and the unlabelled antibodies are competeting for the antigen under 2). Finally, the kit presupposes that the reagents under 2) described in the two arrangements are present in such amount and distributed in such a way that absence of compounds to be detected in the test specimen will make the reagents under 1) stop at 2).

In order to further describe the kit, the following examples are provided.

EXAMPLE I: TEST FOR HUMAN hCG

The kit comprises a cylinder of 8 mm diameter and 40 mm long (Figure 1). The cylinder is constructed of 4 pieces, each 8 mm diameter and 10 mm long. These pieces are in the order mentioned

1. An empty piece which consists of cellulose as absorbant material.
2. A cellulose piece in which is included purified antibodies linked with enzyme ("FASSEL-antibodies").
3. A cellulose-piece in which is included hCG--linked Sepharose-4B particles.
4. A cellulose piece in which is included the substrate.

In the above-mentioned example, the antibody is a monoclonal mouse hybridoma-antibody which is

thoroughly purified and linked with alkaline phosphatase in such a way that no enzyme is free. The substrate consists of lyophilized 5-bromo-4-chloro-3 indolyl phosphate (5-BCIP) in mixture with nitro blue tetrazolium. The antibodies and hCG-particles are present in such amounts that the occurrence of at least 50 IU hCG/l test fluid will produce a blue colour at the end of piece 4 when the end of piece 1 is introduced in the test fluid until fluid is sucked through the kit by capillary forces to the extremity of piece 4. Less than this give negative results.

EXAMPLE II. TEST FOR HUMAN LUTEINISING HORMONE (LH)

The kit comprises a cylinder of the same size and consisting of 4 pieces as described under EXAMPLE I. The pieces are in the order mentioned

1. An empty piece which consists of cellulose as absorbant material.
2. A cellulose piece in which is included purified antigen linked with enzyme (alkaline fosfatase). The antigen is human LH.
3. A piece in which is made a single channel in the middle of 1/2 mm whereas the rest of the cylinder consists of hydrophobic plastic. In the channel is Sepharose 4B to which is linked rabbit antibodies from a commercial polyclonal serum produced by immunizing rabbits with LH.
4. A substrate piece as described under EXAMPLE I.

Thus, the invention comprises:

- A method and kit for qualitative and quantitative analysis of antigens, antibodies and/or microorganisms or other cells, which comprizes the following reagents arranged in the following order in the absorbant material and with the reagents in dry alternative lyophilized form: (1) purified complexes of enzyme and antibodies directed against the antigen to be detected; (2) antigen identically to or antigenically related to the

antigen to be detected by the kit and which antigen is immobilized in the absorbant material e.g. by coupling it to particles; and (3) substrate to the enzyme.

- A method according to the invention is carried out by introducing the material to be tested in liquid at (1) and then allowing the liquid to be sucked by capillary forces through step (2) and (3), whereby the presence of the antigen to be detected will prevent the enzyme-labelled antibodies to be attached to step (2) and thereby proceed to step (3) resulting in the degrading of the substrate to colour product by the enzyme, which colour product are visible to the naked eye whereas the absence of the antigen in the test specimen will result in the attachment of all enzyme-labelled antibody to step (2) and the failure of colour to appear at step 3.

- The compound to be detected may be an antibody which by occupying the antigen under the above step (2) will prevent the enzyme-labelled antibody from attaching to the immobilized antigen.

- A kit according to the invention comprises the following reagents arranged in the order given: (1) purified complexes of enzyme and antigen identical with, or antigenically related to the antigen to be detected; antibodies directed against the antigen to be detected by the kit which antibodies are immobilized in the absorbant material e.g. by coupling them to particles; and (3) substrate to the enzyme.

- A method in which equipment for quantitation of the colour changes by spectrophotometry is attached to the substrate step.

- Antibodies for use in the invention may be raised in animals by immunization with the antigen to be detected which raised antibodies then can be obtained through collection of the polyclonal antibodies from the sera or through production of so-called monoclonal antibodies according to hybridoma technique.

CLAIMS

1. A kit for qualitative and/or quantitative analysis of antigens, antibodies and/or microorganisms or other cells, comprising a stack of absorbent material in which the following reagents are absorbed in different layers:

(1) purified complexes of enzyme and antibodies directed against the antigen to be detected;

(2) antigen identical with or antigenically related to the antigen to be detected, immobilized in the absorbent material, e.g. by coupling to particles; and

(3) a substrate for the enzyme;

whereby the layers (1) and (2) can be arranged in any order in relation to each other.

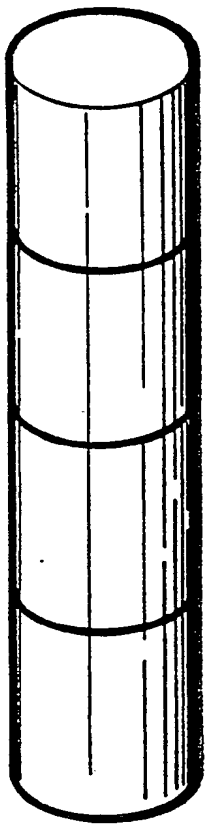
2. A method for qualitative and/or quantitative analysis of antigens, antibodies and/or microorganisms or other cells whereby a solution of the material to be tested is made to pass by capillary forces through a stack of absorbent material, in which the following reagents are absorbed:

(1) purified complexes of enzyme and antibodies directed against the antigen to be detected;

(2) antigen identical with or antigenically related to the antigen to be detected, immobilized in the absorbent material, e.g. by coupling to particles; and

(3) a substrate for the enzyme;

whereby the layers (1) and (2) can be arranged in any order in relation to each other.



INTERNATIONAL SEARCH REPORT

International Application No PCT/SE88/00011

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) *

According to International Patent Classification (IPC) or to both National Classification and IPC 4

G 01 N 33/543, 33/538

II. FIELDS SEARCHED

Minimum Documentation Searched 7

Classification System	Classification Symbols
IPC 3	G 01 N 33/53, /538, /543, /544, /545
US Cl	435:5-7; 436:500, 514, 515, 518, 528-535

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched 8

SE, NO, DK, FI classes as above

III. DOCUMENTS CONSIDERED TO BE RELEVANT 9

Category *	Citation of Document, 11 with indication, where appropriate, of the relevant passages 12	Relevant to Claim No. 13
X	Teknisk Utblick, no. 6, 1985, p. 12, see left col. second part.	1,2
X	GB, A, 2 111 676 (LANCE ALLEN LIOTTA) 6 July 1983 & FR, 2514511 DE, 3237046 BE, 894662 SE, 8205751 JP, 58076763 NL, 8203946 LU, 84402 US, 4446232 CA, 1200483	1,2
X,E	EP, A2, 0 249 851 (MILES LABORATORIES, INC.) 23 December 1987 & JP, 63003263	1,2
X	EP, A1, 0 173 375 (POLAROID CORPORATION) 5 March 1986 & JP, 61038563	1,2

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
1988-04-11	1988 -04- 15
International Searching Authority	**Signature of Authorized Officer**
Swedish Patent Office	Carl Olof Gustafsson

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

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A	Patent Abstract of Japan, Vol. 8, No. 147(P-285), abstract of JP 59-44660, publ. 1983-03-13	1
A	Patent Abstract of Japan, Vol. 8, No. 68(P-264), abstract of JP 58-214855, publ. 1983-12-14	1,2
A	EP, A1, 0 225 054 (BOOTS-CELLTECH DIAGNOSTICS LIMITED) 10 June 1987	1,2
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A	EP, A1, 0 209 378 (EASTMAN KODAK COMPANY) 21 January 1987	1,2